*Trans*IT®-3T3 Transfection Kit

Product Name	Volume of *Trans*IT®-3T3 Reagent	Volume of 3T3 Authority Reagent	Product No.
*Trans*IT®-3T3 Transfection Kit	0.4 ml	0.2 ml	MIR 2184
	1 ml	0.5 ml	MIR 2180
	5 ml (5 x 1 ml)	2.5 ml (5 x 0.5 ml)	MIR 2185
	10 ml (10 x 1 ml)	5 ml (10 x 0.5 ml)	MIR 2186

1.0 DESCRIPTION

1.1 General Information

The *Trans*IT®-3T3 Transfection Kit was developed by the nucleic acid delivery specialists of Mirus Bio Corporation. This novel kit was specifically optimized to provide superior transfection efficiency in 3T3 cells without sacrificing cellular health. The specificity of the *Trans*IT®-3T3 Transfection Kit makes this product a desirable alternative to broad spectrum transfection reagents. The kit provides all the attributes of the trusted *Trans*IT® Reagent line: high efficiency, low toxicity, simplicity of use, and reproducibility. The *Trans*IT®-3T3 Transfection Kit is quality control tested on ATCC 3T3 cells. MIR 2180 provides sufficient amounts of both reagents to perform up to 500 transfections per well in a 6-well plate.

1.2 Specifications

Concentration: *Trans*IT®-3T3 Reagent: 1.33 mg/ml in 80% ethanol
3T3 Authority Reagent: 2 mg/ml in 80% ethanol

Storage: Store both reagents at -20˚C. Prior to use, warm the *Trans*IT®-3T3 Reagent and the 3T3 Authority Reagent to room temperature and gently vortex to dissolve any precipitate that may have formed.

Stability: 6 months from the date of purchase when stored at -20˚C

2.0 PROCEDURE

2.1 Transfection Optimization

The key to successful transfection is careful optimization of reaction conditions for each 3T3 subtype. The transfection protocols described in Section 3.2 should result in efficient transfection of most 3T3 cell subtypes; however, to ensure optimal results the following variables should be considered:

A. Media conditions - *Trans*IT® Reagents yield improved transfection efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) without a media change.

B. Cell density (% confluence) at transfection - The recommended cell density for most 3T3 cell subtypes at transfection is 50-80% confluence. Determine the optimal cell density for each cell type in order to maximize transfection efficiency. Maintain this density in future experiments for reproducibility.

C. DNA purity and concentration for transfection - DNA used for transfection should be highly purified, sterile, and free from contaminants such as endotoxin. Remove any traces of endotoxin (bacterial lipopolysaccharides) using the MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900). The optimal DNA concentration for transfection is 1-5 µg per well of a 6-well plate. As a starting point, use 2.5 µg per well of a 6-well plate.

D. ***Trans*IT®-3T3 Reagent to DNA ratio -** As a starting point, use 3 µl of *Trans*IT®-3T3 Reagent per 1 µg of DNA. Titrate the *Trans*IT®-3T3 Reagent from 2-6 µl per 1 µg DNA. For future transfections, use the ratio that gives the best transfection efficiency with the lowest cellular toxicity, on similarly passaged cells. Refer to Table 1 for recommended starting conditions.

E. **3T3 Authority Reagent to DNA ratio -** As a starting point, use 1 µl of 3T3 Authority Reagent per 1 µg of DNA. The optimal 3T3 Authority Reagent to DNA ratio can be determined by titrating the reagent from 0.5-3 µl per µg DNA. The ratio that gives the best transfection efficiency with the lowest cellular toxicity should be used for future transfections on similarly passaged cells. Refer to Table 1 for recommended starting conditions.

F. **Transfection incubation time -** Determine the optimal incubation time empirically by testing a range from 4-48 hours post-transfection. Mirus Bio recommends an incubation time of 24-48 hours for most applications.

The protocols below are recommended for performing transfections in 6-well plates. When performing transfections in different sized wells, the amount of DNA, *Trans*IT®-3T3 Reagent, 3T3 Authority Reagent, and medium should be scaled up or down in proportion to the surface area of the well. To minimize pipetting small volumes, dilute the *Trans*IT®-3T3 Reagent in 80% ethanol immediately prior to each use. The 3T3 Authority Reagent can be diluted in sterile water immediately before use. Only dilute the required amount of 3T3 Authority Reagent. DO NOT store diluted 3T3 Authority Reagent.

Table 1. Recommended starting conditions for using the *Trans*IT®-3T3 Transfection Kit:

Culture Vessel	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate	10 cm dish
Surface Area*	0.35 cm^2	1.0 cm^2	1.9 cm^2	3.8 cm^2	9.6 cm^2	59 cm^2
Serum-free Media	9 µl	26 µl	50 µl	100 µl	250 µl	1.5 ml
*Trans*IT®-3T3 Reagent	0.28 µl	0.79 µl	1.5 µl	3 µl	7.5 µl	45 µl
DNA (1µg/µl stock)	0.1 µl	0.26 µl	0.5 µl	1 µl	2.5 µl	15 µl
3T3 Authority Reagent (2 µg/µl stock)	0.1 µl	0.26 µl	0.5 µl	1 µl	2.5 µl	15 µl
Complete Growth Media	0.092 ml	0.263 ml	0.500 ml	1.0 ml	2.5 ml	15.5 ml

*Surface areas are based on Greiner tissue culture plates and Falcon 10 cm dishes. All volumes in Table 1 are per one well of indicated size.

2.2 Protocol for Transient Transfection (in 6-well Plates)

A. Cell Plating

1. Approximately 24 hours prior to transfection, plate cells at an appropriate cell density (~1-3x 10^5 cells in complete growth medium per well) to obtain ~50-80% confluence the following day. [a]
2. Incubate the cells overnight.[b]

B. Complex Formation (perform this procedure immediately prior to transfection)

1. In a sterile plastic tube, add the *Trans*IT®-3T3 Transfection Reagent (3 µl per 1 µg DNA) directly into 250 µl of serum-free medium.[c] Mix completely by gentle pipetting.
2. Add plasmid DNA (2.5 µg) to the diluted *Trans*IT®-3T3 Reagent and mix by gentle pipetting.[d]
3. Add 3T3 Authority Reagent (1 µl per 1 µg of DNA) to the diluted *Trans*IT®-3T3/DNA Reagent complexes and mix by gentle pipetting.
4. Incubate at room temperature 15-30 minutes.

C. Cell Preparation for Transfections in Complete Growth Medium

NOTE: The *Trans*IT®-3T3 Transfection Kit yields improved transfection efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) without a media change.

1. If necessary, remove the medium from the cells prepared in step A and replace it with 2.5 ml per well of fresh complete growth medium.
2. Add the *Trans*IT®-3T3 Reagent/DNA/3T3 Authority Reagent complex mixture prepared in step B dropwise to the cells in complete growth medium. Gently rock the dish back and forth and from side to side to distribute the complexes evenly.
3. Incubate for 24-48 hours. [b]

   NOTE: The above incubation is designed for transfections performed with no media change. To perform a media change after a 4-24 hour incubation with the complexes, replace the original medium with fresh complete growth medium and incubate for an additional 24-48 hours.[b,e]
4. Harvest cells and assay as needed.

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[a] Since the optimal cell density (confluence) for efficient transfection can vary between 3T3 cell subtypes, maintain the same seeding protocol for subsequent experiments.

[b] Standard incubation conditions for 3T3 cells are 37˚C in 5% CO_2.

[c] The *Trans*IT®-3T3 Reagent/DNA/3T3 Authority Reagent complex may form improperly if the complex formation medium contains serum, resulting in poor transfection efficiencies.

[e] The optimal incubation time can be determined empirically by testing a range of incubation times from 4-48 hrs.

[d] For transfecting larger amounts of DNA, or if a precipitate forms upon adding the reagent, increase the volume of serum-free medium to 200-1,000 µl.

3.0 TROUBLESHOOTING

Low Transfection Efficiency

- **Suboptimal *Trans*IT®-3T3 Reagent to DNA ratio**
  Determine the optimal *Trans*IT®-3T3 Reagent to DNA ratio by titrating the reagent from 2-6 µl per µg DNA. Choose the amount which gives the best transfection efficiency and the lowest cellular toxicity. As a starting point, use 3 µl of *Trans*IT®-3T3 Reagent.
- **Suboptimal amounts of 3T3 Authority Reagent**
  Try increasing the amount of 3T3 Authority Reagent in small increments from 0.5-3 µl, whereby an ideal concentration of reagent is reached with maximal transfection efficiency and minimal cellular toxicity. As a starting point, use 1 µl of 3T3 Authority Reagent.
- **Cell density (% confluence) not optimal at time of transfection**
  The recommended cell density for most 3T3 cell types at the time of transfection is 50-80% confluence. However, it may be necessary to determine the optimal cell density for specialized experiments in order to maximize transfection efficiency. Maintain this density in future experiments for reproducibility.
- **Poor quality of transfecting DNA (DNA may be partially degraded or an inhibitor, such as an endotoxin, may be present in the preparation)**
  DNA used for transfection should be highly purified, sterile, and free from contaminants such as endotoxin. Remove any traces of endotoxin (bacterial lipopolysaccharide) using the MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900). The optimal DNA concentration for transfection is 1-5 µg per well of a 6-well plate. As a starting point, use 2.5 µg per well of a 6-well plate.
- **Serum present during *Trans*IT®-3T3 Reagent/DNA/3T3 Authority Reagent complex formation**
  Use serum-free medium when forming the complexes. The complexes should be added to cells in their normal complete growth media.
- **Inhibitor present during transfection**
  The presence of polyanions, such as dextran sulfate or heparin, can inhibit transfection. Use transfection medium that does not contain these polyanions.

High Cellular Toxicity

- **Cell density (% confluence) not optimal at time of transfection**
  The recommended cell density for most 3T3 cell types at the time of transfection is 50-80% confluence. However, it may be necessary to determine the optimal cell density for specialized experiments in order to maximize transfection efficiency. Maintain this density in future experiments for reproducibility.
- **Complexes were added to the cells in serum-free media**
  Form complexes in serum-free media, and add to cells in complete growth media (serum containing). Transfection efficiency is improved and cytotoxicity is decreased when the complexes are added to cells in complete growth media.
- ***Trans*IT®-3T3 Reagent/DNA/3T3 Authority Reagent complex mixture was not mixed thoroughly following addition to the cells**
  Mix thoroughly to evenly distribute the complexes to all cells. Rocking the dish back and forth and from side to side is recommended. Do not swirl or rotate the dish, as this may result in uneven distribution.
- **Excessive amounts of *Trans*IT®-3T3 Reagent, DNA, or 3T3 Authority Reagent complex mixture were used in transfection**
  Reduce the amount of appropriate reagent or DNA in the transfection. See Table 1 for recommended starting conditions.
- **Cell morphology has changed**
  If the passage number of the cells is too high or too low, they can be more sensitive to transfection reagents. We recommend maintaining a similar passage number between experiments to ensure reproducibility.

For specific questions or concerns, please contact Mirus Bio Technical Support at 888.530.0801 or techsupport@mirusbio.com.
For a list of citations using Mirus Bio products, please visit the Technical Resources section of our website at www.mirusbio.com.

4.0 RELATED PRODUCTS

For endotoxin removal from DNA:*

MiraCLEAN® Endotoxin Removal Kit (Product #5900)

For DNA tracking studies:

Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit (Product # MIR 7010,7011,7012,7013,7014,7015)

Label IT® Plasmid Delivery Control, Cy™3 or Fluorescein, (Product # MIR 7904, 7905, 7906, 7907)

For determination of gene expression efficiency:

Beta-Gal Staining Kit (Product # MIR 2600)

Transfection reagents:*

*Trans*IT®-LT1 Transfection Reagent (Product # MIR 2300)
*Trans*IT®-LT2 Transfection Reagent (Product # MIR 2400)
*Trans*IT®-Express Transfection Reagent (Product # MIR 2000)
*Trans*IT®-HeLaMONSTER® Transfection Kit (Product # MIR 2900)
*Trans*IT®-Keratinocyte Transfection Reagent (Product # MIR 2800)
*Trans*IT®-CHO Transfection Kit (Product # MIR 2170)
*Trans*IT®-3T3 Transfection Kit (Product # MIR 2180)
*Trans*IT®-293 Transfection Kit (Product # MIR 2700)
*Trans*IT®-COS Transfection Kit (Product # MIR 2190)
*Trans*IT®-Insecta Transfection Reagent (Product # MIR 2200)
*Trans*IT®-Jurkat Transfection Reagent (Product # MIR 2120)
*Trans*IT®-Prostate Transfection Kit (Product # MIR 2130)
*Trans*IT-Neural® Transfection Reagent (Product # MIR 2140)
*Trans*IT®-mRNA Transfection Kit (Product # MIR 2250)
*Trans*IT-TKO® siRNA Transfection Reagent (Product # MIR 2150)
*Trans*IT®-siQUEST™ siRNA Transfection Reagent (Product # MIR 2110)
*Trans*IT®-Oligo Transfection Reagent (Product # MIR 2160)

In Vivo* Gene Delivery Kits:

*Trans*IT®-*In Vivo* Gene Delivery System (Product # MIR 5100)
*Trans*IT®-EE Hydrodynamic Delivery Solution (Product # MIR 5340)
*Trans*IT®-EE Hydrodynamic Delivery Starter Kit (Product # MIR 5310)
*Trans*IT®-QR Hydrodynamic Delivery Solution (Product # MIR 5240)
*Trans*IT®-QR Hydrodynamic Delivery Starter Kit (Product # MIR 5210)

RNA Interference Products:*

*Trans*IT-TKO® siRNA Transfection Reagent (Product # MIR 2150)
*Trans*IT®-siQUEST™ siRNA Transfection Reagent (Product # MIR 2110)
siXpress® PCR Vector Systems (Product # MIR 7300, 7301, 7302)
Label IT®siRNA Tracker Intracellular Localization Kit with *Trans*IT-TKO® Transfection Reagent (Product # MIR 7200,7201,7202,7203,7204,7205)
Label IT®siRNA Tracker Intracellular Localization Kit with *Trans*IT®-siQUEST™ Transfection Reagent (Product # MIR 7206,7207,7208,7209,7210,7211)
Label IT®siRNA Tracker Intracellular Localization Kit (Product # MIR 7212,7213,7214,7215,7216,7217)

*These products are available in additional sizes.